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13. ABSTRACT ***(Maximum 200 Words)***

We have developed a vaccine based on the fusions of DC with tumor cells. The fusion cells express MHC class I and II, costimulatory molecules and tumor-derived peptides. Immunization with dendritic/tumor fusion cells (FC/MUC1) prevented MC38/MUC1 tumor cell challenge and eradicated established MUC1-positve pulmonary metastasis in MUC1 transgenic mice (MUC1.Tg). In the present study, MUC1 transgenic mice are crossed with strains that express polyoma middle T antigen (MTag) driven by the mouse mammary tumor virus (MMTV-LTR) promoter. The new breeder (MMT mice) develops spontaneous mammary carcinoma. We demonstrate that MMT or MT mice form mammary carcinoma with three stages: (i) latent stage from new born to 3 weeks old. (ii) premalignant stage from age of 4 weeks to 5 weeks. (iii) stage of carcinoma formation from age of 6 weeks or older. Immunization with DC/tumor fusion cells in the premalignant stage rendered the 78% mice free of disease. These results match our hypothesis that immunization with fusion cells can generate an effective antitumor response to block the progression of premalignant lesion in MMT mice.

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Prevention and Treatment of Spontaneous Mammary Carcinoma with Dendritic-tumor Fusion Cell Vaccine

Annual Report
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Grant Number DAMA17-00-1-0220
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INTRODUCTION:

MUC1 transgenic mice are crossed with strains that express polyoma middle T antigen (MTag) driven by the mouse mammary tumor virus (MMTV-LTR) promoter. The new breeder (MMT mice) develops spontaneous mammary carcinoma. The proposed study develops a hybrid cell vaccine by fusing dendritic cells (DC) with MUC1 expressing carcinoma cells. Furthermore, the effectiveness of fusion cell vaccination in the prevention and treatment of spontaneous mammary carcinoma are investigated in MMT mice in which the mammary glands develop spontaneous carcinoma in a way similar to that of humans.

BODY:

1. Generation of MMT and MT mice

The male Mtag mice are crossed with female MUC1.Tg mice to generate MMT mice. Routine polymerase chain reaction (PCR) is performed to identify the new born with expression of Mtag and MUC1 antigens. As a control, Mtag mice are crossed with wild-type C56BL/6 mice to generate mice expressing Mtag (MT). Both MMT and MT mice develop spontaneous mammary carcinomas.

Generation of MMT mice for experiment is a slow process since less than _ new breeders expressing both Mtag and MUC1 and only female mice are suitable for experiment.

2. Characterization of spontaneous mammary tumorigenesis in MMT and MT mice.

Groups of female MMT or MT mice (3 in each group) were sacrificed at age of 3, 4, 6, 8 and 10 weeks to determine the pattern of mammary carcinoma formation under microscope. At age of 3 weeks, very small mammary gland can be obtained. These glands look normal under microscope. At age of 4 weeks, the appearance of dysplasia of mammary epithelia was demonstrated. At age of 6 weeks or older, all the mice examined exhibited the formation of mammary carcinoma. These results indicate that MMT or MT mice form mammary carcinoma with three stages: (i) latent stage from new born to 3 weeks old. (ii) premalignant stage from age of 4 weeks to 5 weeks. (iii) stage of carcinoma formation from age of 6 weeks or older.

Female MMT or MT mice were palpated weekly starting at age of 6 weeks for tumor development. Most mice developed multiple and visible mammary carcinomas at age of 8 -10 weeks. The tumors progressed very rapidly. The mice become moribund about 16-20 weeks. There was no difference of mammary carcinoma development between MMT and MT mice.

3. Immunization with dendritic/tumor fusion cells stop progress of premlignant lesion into mammary carcinoma in MMT and MT mice.

In the first set of experiments, 18 MMT mice were immunized with FC/MUC1 (DC fused with MC38/MUC1 adenocarcinoma cell line) for four times starting at age of 30 days (mice in the premalignant stage), then the immunization was repeated every other week. Nine mice (50%) were survived up to 12 months free of disease.

To improve the effectiveness of vaccine, in the ongoing studies, DC were fused with carcinoma cells isolated from MMT mice. MMT mice (N=18) are immunized five times (at age of 30, 45, 60, 75 and 90 days) with DC/MMT tumor fusion cells. The mice are followed for 26-30 weeks now. Fourteen mice (78%) still survive free of disease. The mice will be followed for up to 12 months. These studies indicate that early vaccination with DC/MMT fusion cells prevents or stops the tumorigenesis process in MMT mice.

DISCUSSION:

We have developed a vaccine based on the fusions of DC with tumor cells. The fusion cells express MHC class I and II, costimulatory molecules and tumor-derived peptides. They are well equipped to activate T cells in the right environment (appendix 1). In our model, we demonstrate that immunization with DC/tumor fusion cells in the premalignant stage rendered the 78% mice free of disease. These results match our hypothesis that immunization with fusion cells can generate an effective antitumor response to block the progression of premalignant lesion in MMT mice.

In the coming year, we will continue the prevention and treatment of mammary carcinomas by DC/MMT or DC/MT fusion cell immunization in MMT or MT mice. We will immunize the MMT or MT mice in the latent stage (3 weeks old or younger) although it is technically challenge. In the treatment, considering the tumor burden, immunization with fusion cells will be combined with other antitumor agents or treatment modalities to enhance the antitumor effect.

CONCLUSIONS:

1. The tumorigenesis of MMT or MT mice has been characterized.
2. Immunization with DC/MMT fusion cells are effective to block the premalignant lesion progressing into mammary carcinoma.

PUBLICATIONS

1. Shigeo Koido, Yasuhiro Tanaka, Dongshu Chen and Jianlin Gong. The Kinetics of in Vivo Priming CD4 and CD8 T Cells by Dendritic/Tumor Fusion Cells in MUC1

Transgenic Mice. (Submitted for publication).